FINAL REPORT

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A System and Methodology for the Measurement of Volatile Organic Compounds Produced by Hydroponic Lettuce in a Controlled Environment

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The National Aeronautics and Space Administration (NASA) conducts and supports research toward the development of plant cultivation techniques for life support systems in space, specifically through the Controlled Ecological Life Support System (CELSS) program. Among the challenges in the development of a viable plant production system in a closed environment is the management of potentially toxic trace gases emitted by plants. In order to assess possible dangers that volatile plant chemicals might pose to the safety of a space crew, the qualitative and quantitative determinations of these plant emissions are essential.

Differential pressure measurements obtained during the transition from the light to the dark period did not vary significantly during the temperature change from 23 C to 18 C. This result was consistent with the fact that although the air within the glass jar was pressurized relative to external air, there was a significant amount of leakage at each of the three ports on the top of the jar and around its base where it was supported by the aluminum plate. Backflow of contaminants into the jar was prevented by this leakage.

The flow of purified air into the glass jar purged the jar 1-1.5 times/min. This continual flushing of the jar minimized accumulation of vapor phase volatile products and the consequent formation of a gradual chemical potential gradient around the leaves. The continual evacuation of airborne compounds may enhance volatile emission rates somewhat by increasing the chemical gradient at this interface.

Occurrence and Identification of Lettuce Volatiles

Analyses of air samples collected during the 2-h period immediately after the end of the light period consistently indicated the presence of three compounds beginning at 21 DAT. None of these compounds were detected during other periods of the diurnal cycle. In order to confirm that the compounds detected originated from the lettuce and not from the collection system, chromatograms representing background collections in which the glass jar was empty were compared to those from collections in which the jar was placed over a lettuce plant.

The compounds were identified as (Z)-3-hexenal, (Z)-3-hexenol, and (Z)-3-hexenyl acetate by comparing their mass spectra with those of authentic compounds. Both the natural products and authentic compounds were analyzed with the mass spectrometer operating in the electron impact and chemical ionization modes. Additional confirmation of the identification of the plant compounds was obtained by comparing their GC retention times with those of authentic chemical standards. The relative amounts of each plant compound are listed in.

The system and methodology employed in this study detected (Z)-3-hexenal, (Z)-3-hexenol, and (Z)-3-hexenyl acetate, but could not detect ethylene. The emission of ethylene from lettuce is currently being researched by NASA at the John F. Kennedy Space Center. We did not find any literature describing additional volatiles measured from undisturbed lettuce plants.

(Z)-3-hexenol and (Z)-3-hexenyl acetate were the most dominant oxygenated hydrocarbons emitted from over 30 agricultural and natural plant species surveyed. (Z)-3-hexenol and (Z)-3-hexenyl acetate were detected from lettuce under field conditions but did

not report any effects of environment on emission rates. (Z)-3-hexenal has been detected in emissions from tomato (*Lycopersicon esculentum* L.) leaves. The headspace of juice removed from lettuce with a seive and extractor contained the isopropyl, sec-butyl, and isobutyl forms of 3-alkyl-2-methoxypyrazine. The presence of these compounds in headspace samples over undamaged lettuce has not been reported.

Volatiles Emitted by Damaged Lettuce

Although (Z)-3-hexenal, (Z)-3-hexenol, and (Z)-3-hexenyl acetate were only detected immediately following the end of the light period under normal sampling conditions, damaged lettuce emitted these compounds regardless of whether the damage was inflicted during the light or the dark period. Bruising a head of lettuce resulted in highly elevated emissions of (Z)-3-hexenol and increased emissions of (Z)-3-hexenol and (Z)-3-hexenyl acetate were detected from agricultural crops subjected to "rough handling." Lipoxygenase pathway products such as (Z)-3-hexen-1-ol, hexanol, (E)-2-hexenal, (Z)-3-hexenal, and hexanal commonly evolve from macerated green plant material.

(Z)-3-hexenal and (Z)-3-hexenol exhibit antifungal effects against *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Didymella lycopersici*, and *Cladosporium fulvum*. The release of these compounds in response to mechanical stress may function as a type of defense mechanism. Other structurally similar C_6 compounds have antibacterial properties. (Z)-3-hexenal, (Z)-3-hexenol, and (Z)-3-hexenyl acetate may be of concern in a closed system such as CELSS under development by the NASA. In earth's atmosphere, (Z)-3-hexenol and (Z)-3-hexenyl acetate have tropospheric lifetimes of 1-3 h. They react with OH and NO_3 radicals and ozone to produce propanal, 3-hydroxypropanal, and 3-acetoxypropanal. Subsequent chemical conversions can lead to peroxypropionyl nitrate (PPN) and peroxyacetyl nitrate (PAN). The fate of (Z)-3-hexenol and (Z)-3-hexenyl acetate should be investigated in a CELSS environment.

Volatile emission rates are influenced by the resistance within the diffusive pathway from biosynthetic sites to the atmosphere. The physical distance and path traveled by (Z)-3-hexenal, (Z)-3-hexenol, and (Z)-3-hexenyl acetate are factors which could affect emissions. The physical path has not been clearly elucidated but probably would involve diffusion through cells, intercellular air spaces, passage through the stomata, and perhaps through the cuticle as well.

Relative humidity is another factor that may affect volatile emissions from lettuce. Relative humidity was not actively controlled in the sampling jar. Due to the condensation effects from passing through the coil immersed in ice water, the purified air entering the glass jar had low relative humidity. With no plant or when a lettuce seedling was present inside the jar, relative humidity typically ranged from about 15% to 30%. As the plant increased in biomass, relative humidity values increased to 50% to 60%.

When exposed to continuous light for a 72-h period beginning at 32 DAT, no volatiles were collected until the dark cycle was restored. This result suggests that the emission of C_6 compounds from lettuce at the onset of the dark period is not a circadian rhythm. Periodic emissions that are circadian in nature would continue, at least for some time, under constant illumination. Future research should investigate whether the plant

emissions in a CELSS environment could be reduced by growing plants under continuous light from planting to harvest.

Lettuce Growth Rate Comparison Between Chambers

The growth of lettuce in growth chamber model E15 was compared to that in growth chamber model EF7, the modified growth chamber designed for volatile collections. The mean relative growth rates for shoot fresh weight and shoot dry weight for each chamber were calculated as follows:

$$Mean\ RGR = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1}$$

where mean RGR = mean relative growth rate ($mg \cdot g^{-1} \cdot d^{-1}$), the increase in biomass per unit of biomass present per unit of time. $\ln(W_2)$-$\ln(W_1)$ = difference between natural logarithms of shoot fresh or dry weights at times t_2 and t_1 (days), and t_2-t_1 = time interval between harvests (days). In both growth chambers, highest mean RGR was measured at 21 DAT. Thus the rate of accumulation of dry matter relative to the existing dry matter was highest at this time. The general trend of mean RGR calculated on a shoot fresh weight basis decreased somewhat at 28 DAT before increasing again at 35 DAT, whereas mean RGR for shoot dry weight continually decreased after 21 DAT.

Mean relative growth rates for shoot fresh weight and shoot dry weight were not significantly different between growth chambers at any specified time. Moreover, on a fresh weight basis, there were no signficant differences between any mean RGR values with the exception of chamber E15 at 21 DAT. The mean RGR values based on shoot dry weight were not significantly different between growth chambers at any specified time. No differences were detected between mean RGR values of either chamber from 14 to 21 DAT, or from 28 to 32 DAT. These data indicate that growth trends were not significantly different between growth chambers EF7 and E15. Therefore volatile emission rates could be calculated from planting to harvest based on measurements of volatiles from chamber E7 and measurements of biomass from E15.

The nondestructive sampling of volatile compounds emitted by plants was accomplished in the controlled environment of a growth chamber. Lettuce plants were undisturbed by the methodology employed. Consequently, volatiles were collected from plants up to 32 DAT. The capabilities of this system are valuable for CELSS-related research in that environmental conditions can be controlled and volatile emissions measured over the growth cycle of the plants. Determinations of volatile plant emissions are important as possible indicators of stress responses within plants, for air quality studies, and for investigations of the natural pest resistance provided by certain volatile compounds.